STUDIES ON BOVINE BRUCELLOSIS IMMUNIZATION

CONTROLLED EXPERIMENTS WITH B. A. I. STRAIN 19, HUDDLESON'S MUCOID AND OTHER BRUCELLA VACCINES

BY

HAROLD STEVER BRYAN

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STUDIES ON BOVINE BRUCELLOSIS IMMUNIZATION: CONTROLLED EXPERIMENTS WITH B. A. I. STRAIN 19, HUDDLESON'S MUCOID AND OTHER BRUCELLA VACCINES

Brucella immunization studies have been carried on by many investigators for over 50 years. As yet, the ideal immunizing agent has not been found.

Early studies centered about the injection of living, virulent *Brucella abortus* organisms into cattle. This procedure established a significant degree of resistance against challenging infection but had the disadvantage of producing chronic carriers and spreaders of virulent organisms. The disadvantages so outweighed the advantages that immunization with virulent cultures was finally abandoned. Cultures of *Br. abortus* killed by heat, chemical or physical treatment gave discouraging results. Specific immune serum proved worthless as a protective agent against bovine brucellosis. Soluble and sonic extracts of *Br. abortus* had been used in immunization tests, but without great success.

Huddleson (1924) was the first to report on the use of a living, nonvirulent *Br. abortus* culture for immunization purposes. Subsequently, Buck (1930) reported from the U. S. Bureau of Animal Industry laboratory on the use of a *Br. abortus* culture of low virulence. This culture, designated as B. A. I. strain 19, induced a significant resistance in cattle. It was regarded as the best immunizing agent available when this study was initiated in 1948. One of the main disadvantages of strain 19 vaccine is that it causes a disturbing number of animals to give fluctuating, suspicious reactions in the serum agglutination test. These reactions may persist a long time.

The use of dissociated growth phases of Brucella in the preparation of vaccines was initiated by Huddleson (1946). The low pathogenicity and the low agglutinin titer response which characterized the M (mucoid) growth phases of *Br. suis* in guinea pig experimental work led him to the preparation of *Br. suis* mucoid vaccine for use in cattle. When the present

study began in 1948, it was known that Huddleson's mucoid vaccine did not produce a high or persistent agglutinin titer, but little or no information was available on the degree of resistance, if any, it induced in cattle.

Two experimental Brucella vaccines were prepared for use in this investigation. One (Illinois 2012-5M) was prepared from a dissociated strain (roughlike type) of *Br. suis* and the second (Illinois 2012-50SK) was a bacterin prepared by streptomycin inactivation of a smooth culture of *Br. suis*.

The purpose of this investigation was to determine the protective value of B. A. I. strain 19, Huddelson's mucoid and the two experimental vaccines in immunizing cattle against brucellosis.

Five experiments involving 228 heifers vaccinated between the ages of 4 and 8 months were completed. All heifers were Herefords except 12, which were Holstein-Friesians.

The resistance of the heifers to Brucella infection was challenged during the mid-gestation period of the first pregnancy by conjunctival sac instillation of virulent *Br. abortus* strain 2308 organisms. The exposure dose varied with the experiment from 4 million to 14 million viable organisms per heifer.

The protective value of the vaccines was appraised by (1) abnormal births, (2) recovery of *Br. abortus* and (3) positive serum agglutination tests after challenge of the pregnant heifers. The results of four experiments with a total of 139 pregnant heifers were homogeneous statistically and served as the basis for the conclusions.

Abnormal births occurred in 11 (35 per cent) of 31 strain 19 vaccinates, 27 (79 per cent) of 34 Huddleson's mucoid vaccinates and 22 (88 per cent) of 25 unvaccinated controls.

Recovery of *Br. abortus* by cultural methods and guinea pig inoculation with specimen material from the heifer and/or her aborted fetus was obtained from 12 (46 per cent) of 26 specimens from strain 19 vaccinates, 26 (76 per cent) of 34 specimens from Huddleson's mucoid vaccinates and from 22 (88 per cent) of 25 specimens from the unvaccinated controls.

Positive Brucella serum agglutination tests (titers of

1:100 or higher) were obtained after challenge in 14 (45 per cent) of 31 strain 19 vaccinates, 28 (82 per cent) of 34 Huddleson's mucoid vaccinates and in 22 (88 per cent) of 25 unvaccinated controls.

The experimental vaccine, Illinois 2012-5M, and the experimental bacterin, Illinois 2012-50SK, were tested on 49 pregnant heifers. Neither the vaccine nor the bacterin provided protection against subsequent *Br. abortus* challenge as demonstrated by abnormal births, recovery of *Br. abortus* and positive serum agglutination tests. The results obtained are summarized in Table 1.

The rate of normal, inherent resistance for each group of heifers, as indicated by the failure of infection, was used in determining the protective values of the various vaccines. Based upon abnormal births, 88 per cent of the unvaccinated controls became infected, i. e., aborted, whereas 12 per cent resisted infection. Of the strain 19 vaccinates, 35 per cent became infected (aborted), whereas 65 per cent were protected. Subtracting the 12 per cent inherent resistance from the total protection obtained, one arrives at 53 per cent as the net protection accomplished by strain 19 vaccine. Similarly, the protective value of Huddleson's mucoid vaccine was 9 per cent, and those of the experimental vaccine and bacterin, none (see Table 2).

Based upon recovery of *Br. abortus* from the heifers or their fetuses at the time of calving, the protective value for strain 19 vaccine was 42 per cent, Huddleson's mucoid vaccine 12 per cent, Illinois 2012-5M subcutaneous 6 per cent and Illinois 2012-5M intradermal and Illinois 2012-50SK bacterin none.

TABLE 1

Summary of Results of Brucella Challenge on Pregnant Vaccinated and Unvaccinated Heifers

VACCINE	No. of Heifers	Live Calves		Abortions		Brucella Isolation Maternal and/or Fetus			Pos. Blood Tests	
		No.	%	No.	%	No. Exams.	Isolations No.	%	No.	%
B. A. I. Strain 19	31	20	65	11	35	26	12	46	14	45
Huddleson's Mucoid	34	7	21	27	79	34	26	76	28	82
Illinois 2012-50SK Bacterin	8	0	.....	8	100	8	6	75	8	100
Illinois 2012-5M Intradermal	8	0	.....	8	100	8	8	100	8	100
Illinois 2012-5M Subcutaneous	33	2	6	31	94	33	27	82	31	94
Unvaccinated Controls	25	3	12	22	88	25	22	88	22	88

TABLE 2

Protective Values of Various Brucella Vaccines

VACCINE	No. of Heifers	Per Cent Protection, Based Upon Calving. %	Recovery of Brucella. %	Blood Test. %
B. A. I. Strain 19	31	53	42	43
Huddleson's Mucoid	34	9	12	6
Illinois 2012-50SK Bacterin	8	0	0	0
Illinois 2012-5M Intradermal	8	0	0	0
Illinois 2012-5M Subcutaneous	33	0	6	0

Based upon positive serum agglutination tests (titers of 1:100 or higher) in heifers after Brucella challenge, the protective value for strain 19 vaccine was 43 per cent, that for Huddleson's mucoid vaccine was 6 per cent and those for the experimental vaccine and bacterin, none.

Statistical analysis of the data revealed that strain 19 vaccine afforded a significant protection against brucellosis. In protection against abortions, the difference was significant at the 0.1 per cent level. Huddleson's mucoid vaccine, the experimental vaccine (Illinois 2012-5M) and the experimental bacterin (Illinois 2012-50SK) did not produce significant resistance. Huddleson's mucoid vaccine may be of some value in providing protection, but the probability of the observed results having occurred by chance was about one chance in ten. This is not an acceptable level of significance.

It was concluded that, under the conditions of these experiments, B. A. I. strain 19 vaccine induced a significant degree of resistance against brucellosis, whereas Huddleson's mucoid vaccine and the two experimental vaccines failed.

Other observations made in connection with the Brucella vaccinal immunization study were reported and discussed.

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VITA

The author was born on July 26, 1920, in Bedminster, Pennsylvania. He received his elementary education in a rural school near Perkasie, Pennsylvania. His secondary school education was completed at the Sellersville-Perkasie High School, Perkasie, Pennsylvania, whereupon he matriculated at Michigan State College, East Lansing, Michigan. He was awarded the degree, Doctor of Veterinary Medicine, with honor, from Michigan State College in 1944. From 1944 to 1945 he was a War Food Assistant employed to assist in a demonstrational program for the control of bovine mastitis. In 1945 he was appointed Assistant in the Department of Veterinary Pathology and Hygiene, University of Illinois. He matriculated in the Graduate College there and was awarded the degree, Master of Science in Veterinary Pathology and Hygiene, in 1948. In 1947 he was given the rank of assistant professor, a position he holds at present.

The author is a member of Phi Zeta, Alpha Psi, Sigma Xi, Phi Sigma, Gamma Sigma Delta, American Veterinary Medical Association, Illinois State Veterinary Medical Association and the United States Livestock Sanitary Association. The titles of some of the more significant of his 17 publications follow.

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